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Heavy metal levels in patients with ineffective erythropoiesis

Turan Bayhan[a,*], Şule Ünal[a], Eyüp Çırak[b], Onur Erdem[b], Cemal Akay[b], Orhan Gürsel[c], İbrahim Eker[c], Erdem Karabulut[d], Fatma Gümrük[a]

[a] Department of Pediatric Hematology, Hacettepe University, Ankara 06100, Turkey
[b] Department of Toxicology, Gulhane Military Medical Academy, Ankara 06010, Turkey
[c] Department of Pediatric Hematology, Gulhane Military Medical Academy, Ankara 06010, Turkey
[d] Department of Biostatistics, Hacettepe University, Ankara, Turkey

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ABSTRACT

Objectives: Iron is taken into enterocytes at the duodenum via apical divalent metal-ion transporter 1 protein. Besides iron, divalent metal-ion transporter 1 also transports other divalent metals. We aimed to investigate blood heavy metal levels in patients with ineffective erythropoiesis.
Methods: Blood levels of heavy metals including Pb, Al, Cd, Cr, Co, Cu, and Zn were measured in patients with thalassemia major (TM), thalassemia intermedia (TI), congenital dyserythropoietic anemia (CDA), and age- and sex-matched healthy controls.
Results: Blood samples were obtained from 68 patients (51 patients with TM, 8 with TI, 9 with CDA), and a control group that included 65 volunteers. Patients with TM were found to have lower Al, Pb, and Zn, and higher Cd levels compared with the control group. The patients treated with deferasirox were further analyzed and Pb and Zn levels were found lower compared with the control group.
Discussion: Patients with TM had tendency to have elevated levels of plasma cadmium; however, the median level was not at a toxic level. Increased metal-ion transporter 1 activity may cause heavy metal accumulation, but deferasirox chelation may be protective against heavy metals besides iron.

1. Introduction

Iron absorption from the gastrointestinal (GI) tract is induced in patients with ineffective erythropoiesis (IE), such as in thalassemia major (TM), thalassemia intermedia (TI), and congenital dyserythropoietic anemia (CDA) [1–3]. Iron is absorbed from the GI tract at the duodenum level via a complex interaction and traffic of multiple proteins [4]. Divalent metal-ion transporter 1 (DMT1) is one of the proteins that exists at the apical part of enterocytes and provides entrance of iron into the enterocytes [4,5]. Expression of DMT1 mRNA and protein is stimulated in the event of iron deficiency, hypoxia, and IE, which results with more iron absorption [2,6–8]. DMT1 also transports divalent metal ions such as cadmium (Cd), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn), nickel (Ni), and zinc (Zn), besides iron [5,9,10].

After the transition to an industrial society, exposure to toxic metals became a global health problem. Even in minimal doses, Pb exposure may cause negative influences on neurocognitive development of children and delayed puberty in girls [11,12]. In adults, Pb toxicity may affect cognitive activity and cause hypertension [12]. The hematopoietic system is also a target of Pb intoxication. Pb interferes with heme biosynthesis and influences the generation and function of red blood cells [13]. Cadmium exposure in minimal doses may result in nephrotoxicity [14]. Chronic Cu toxicity is a cause of hepatotoxicity [15]. In patients with CDA, TI, and TM, chronic iron accumulation and hypoxia may affect many systems including the central nervous system, endocrine system, GI system, genitourinary system, similar to heavy metal toxicity. Therefore, heavy metal toxicity might be underestimated in patients with IE. In the literature, a few studies have reported on patients with TI and TM regarding heavy metal levels, but no studies have covered the majority of heavy metal levels in patients with IE [16–18].

In this study, we aimed to evaluate levels of heavy metals in the blood of patients with IE, particularly in TM, based on our hypothesis that heavy metal absorption from the GI tract via DMT1 might be augmented during increased iron absorption, secondary to stimulus of IE.

Abbreviations: CDA, congenital dyserythropoietic anemia; DMT1, divalent metallo protein 1; GI, gastrointestinal; IE, ineffective erythropoiesis; TI, thalassemia intermedia; TM, thalassemia major.

* Corresponding author.
E-mail address: turanbayhan@yahoo.com (T. Bayhan).

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Table 1
Clinical characteristics of patients in comparison with the control group.

		TM	TI	CDA	Total no. of patients with IE	Control	*p* value[a]
Number		51	8	9	68	65	
Age[b] (year)		20.2 (4.5–39)	8.8 (3.4–37)	9 (2.5–14.6)	18 (2.5–39)	16.8 (3–39)	0.71
Male/Female		23/28	3/5	6/3	32/36	33/32	0.73
Cigarette smoke	Yes	17	3	7	27	28	0.72
exposure	No	34	5	2	41	37	
Transfusion	Yes	51	5	6	62		
requirement	No	0	3	3	6		
Chelator use	No	0	2	4	6		
	Deferasirox	46	6	5	57		
	Deferoxamine	4	0	0	4		
	Deferoxamine + deferriprone	1	0	0	1		
Median serum ferritin[b] (ng/dL)		1096 (226–5300)	946.5 (51.3–1370)	1062 (80–1559)	1091.5 (51.3–5300)	25.4 (8–192)	
Median hemoglobin[b] (g/dL)		9.3 (7.4–12.1)	9 (8–12.7)	8.3 (7.4–9.3)	9.2 (7.4–12.7)	13.6 (11.7–17.8)	

TM, thalassemia major; TI, thalassemia intermedia; CDA, congenital dyserythropoietic anemia; IE, ineffective erythropoiesis.
[a] Comparison of total patients with ineffective erythropoiesis and control group.
[b] Median and range.

2. Materials and methods

The study was performed in the Pediatric Hematology outpatient clinics of two university hospitals, Hacettepe University İhsan Doğramacı Children Hospital and Gülhane Military Medical Academy, between December 2014 and April 2015. Patients with CDA, TI, or TM were enrolled in the study. The diagnosis of CDA was confirmed with gene mutation analysis for CDA type I and II. If patients had ferritin levels constantly >1000 ng/dL and were transfused 10 to 15 times, iron chelation therapy was initiated during their follow-up. Patients aged below two years and those with chronic malabsorption disease were excluded. The age limit of two years was defined because the accumulation of heavy metals may take time and heavy metal levels may be already be low in younger patients. The control group was chosen from age- and sex-matched volunteers without any anemia and/or iron deficiency. Cigarette smoke exposure may cause increased blood Cd levels [19], accordingly, this was questioned for each patient and volunteer. Cigarette smoke exposure was accepted positive if patients were active or passive smoker.

At a routine follow-up visit, blood samples were taken for measurement of aluminum (Al), Cd, Co, chromium (Cr), Cu, Pb, and Zn. Samples were taken before transfusion if the patients were under an erythrocyte transfusion program. Venous blood samples were taken in the morning after a 12 h overnight fast.

This study was approved by the local ethics committee of Hacettepe University Faculty of Medicine, and written informed consent was obtained from the patients or their guardians before participation (approval number; GO 13/373 – 02).

2.1. Measurement of blood levels of heavy metals

Cd, Co, Cr, and Pb were analyzed from plasma samples. For these metals, 1-mL blood samples were digested with 3 mL 65% HNO_3 and 0.5 mL 30% hydrogen peroxide (H_2O_2) in a microwave (Milestone mls 1200 mega, High-Performance Microwave Digestion Unit, Shelton, CT, USA) digestion system. The digested samples were filled with double-glass distilled water up to 5 mL. Al, Cu, and Zn were measured in serum samples. Serum samples were analyzed using an Atomic Absorption Spectrometer (Perkin Elmer Analyst 800, MA, USA) (0.5 mL serum was diluted to 2 mL with 1.5 mL 0.2% HNO_3) [20].

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 software (Armonk, NY: IBM Corp). The Mann–Whitney *U* test was used to compare the medians of the two groups, the Kruskal–Wallis test was used to compare the medians of more than two groups. The Chi-square test or Fisher's exact test, where appropriate, was used to compare proportions in different groups. The correlation coefficients and their significance were calculated using Spearman's test. *p* values below 0.05 were accepted as statistically significant. For the multivariate analysis, the possible factors identified with univariate analyses were further entered into the logistic regression analysis to determine independent predictors. A 5% type I error level was used to infer statistical significance. The study received a grant from Hacettepe University Scientific Research Projects Coordination Unit.

3. Results

A total of 68 patients with IE were enrolled in the study, 51 of whom were diagnosed as having TM, 8 had TI, and 9 had CDA. For the control group, heavy metal measurement was performed for 65 volunteers. The median age of the TM group was 20.2 years (range, 4.5–39 years), the TI group was 8.8 years (range, 3.4–37 years), the CDA group was 9 years (range, 2.5–14.6 years), and the control group was 16.8 years (range, 3–39 years). The clinical and laboratory characteristics of the patient and control groups are shown in Table 1.

When we compared patients with IE and the control group, there was no difference for age, sex, and cigarette smoke exposure. Five (62.5%) patients with TI and six patients with CDA (66.6%) were under a chronic erythrocyte transfusion program. The median ferritin level of the last follow-up year for TM was 1096 ng/dL (range, 226–5300 ng/dL), TI was 946.5 ng/dL (range, 51.3–1370 ng/dL), and CDA was 1062 ng/dL (80–1559 ng/dL). Sixty-two patients were on iron chelators and 57 (92%) were being chelated with deferasirox, four (6.5%) with deferoxamine, and one (1.5%) with both deferoxamine and deferriprone (Table 1).

The heavy metal levels of patients and the control group are shown in Table 2. In the TM group Al, Pb, and Zn levels were significantly lower and the Cd level was greater than in the control group (Table 2). There was no significant difference between the TI group and the control group, and only Zn levels were lower in the CDA group compared with the control group ($p = 0.02$). Heavy metal levels were grouped as low (if the lower limit of the metal's normal range was greater than zero), normal, and high; each is presented in Table 3. Among the patients with IE, Al was measured higher than the upper limit of normal in 22% of patients. On the other hand, Al level was higher than the normal range in 43.1% of the control group ($p = 0.006$). In other metals, values above the normal range were less commonly observed (Table 3). Median Cd level was higher in the TM group; however, there was no difference compared with the control group when the proportions were